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First Report of Plasmid-Mediated *qnrA1* in a Ciprofloxacin-Resistant *Escherichia coli* Strain in Latin America▽

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Among 144 ciprofloxacin-resistant *Escherichia coli* isolated in Brazil, one (0.69%) QnrA1-producing isolate was detected. The *qnrA1* gene was associated with IS*CR1*. The QnrA1 determinant was carried on a 41-kb conjugative plasmid, which also carried a FOX-type cephalosporinase encoding gene and a class 1 integron with the *aadB* and *catB3* cassettes. This is the first report of a *qnrA*-carrying isolate in a Latin American country.

The quinolone resistance gene (*qnr*) was first isolated from an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strain in Birmingham, AL, in 1998 (4). Since *qnrA* was characterized, these genes have been reported in *E. coli* and *K. pneumoniae* from different regions (5, 8). Recently, a new *qnr*-type, *qnrB*, was described in a *K. pneumoniae* strain and in other members of the *Enterobacteriaceae* (10). QnrB showed less than 40% amino acid identity with QnrA. Environmental species have been speculated as the likely source of *qnr*-type determinants (8). The *qnr* gene has been mostly carried by conjugative plasmids that carry other resistance genes as well (8, 10). Thus, the spread of *qnrA* is worrisome because it could jeopardize not only the future clinical use of fluoroquinolones but also the use of other unrelated antimicrobial compounds.

From January 2002 to June 2003, a total of 144 ciprofloxacin-resistant *Escherichia coli* (MIC, ≥4 μg/ml) were isolated from 17 hospital-based laboratories throughout Brazil. Only one isolate per patient was included in the study. A summary description of the demographic data, such as the patient's initials, age, gender, hospitalization ward, and underlying conditions, was obtained. The molecular characterization of these isolates by pulsed-field gel electrophoresis showed a great genomic diversity (data not shown).

All isolates were screened for *qnrA* by colony blotting and hybridization methods as previously described (13). The *qnrA* probe was synthesized from a *K. pneumoniae* strain harboring plasmid pMG252 (4) by PCR with the primers QNRF and QNRR (Table 1). Of the 144 strains, only one *qnrA*-carrying isolate (strain 13.52) was detected (0.69%). Strain 13.52 was isolated from a urinary specimen of an 80-year-old female who was hospitalized in Porto Alegre, Brazil. This isolate showed resistance to most β-lactams (except cefepime and carbapenems), all quinolones tested, streptomycin, and chloramphenicol (Table 2) (1). In contrast, this strain was susceptible to gentamicin (MIC, 2 μg/ml), and amikacin (MIC, 4 μg/ml).

PCR amplification and DNA sequencing were performed with the primers listed in Table 1. The amplicons obtained with PCRs were sequenced on both strands using the ABI Prism 377 system (Applied Biosystems, Foster City, CA). The nucleotide sequences were analyzed by using the Lasergene software package (DNASTAR, Madison, WI), and the sequences obtained were compared to sequences available over the internet (http://www.ncbi.nlm.nih.gov/BLAST/).

Investigation of amplicons obtained with primers for *qnr* and adjacent structures demonstrated that strain 13.52 harbored the same *qnrA1* that was originally identified in the plasmid pMG252 carried by a *K. pneumoniae* strain from Birmingham, AL (GenBank accession number AY070235) (4). DNA sequences produced by further PCR and sequencing experiments assembled a 3,942-bp contiguous sequence (GenBank accession number AM295981). Analysis of this sequence revealed that *qnrA* was located in a *sul1*-type integron structure and was embedded downstream of a putative recombinase sequence, *orf513*, known as common region 1 (IS*CR1*) (11). The quinolone resistance gene was located immediately upstream of a truncated version of *qacE*Δ*1*/*sul1*. This element showed 100% homology to the *sul1*-type integron structure identified in pMG252 (GenBank accession number DQ831140) (7).

Amplification experiments using primers targeting the conserved regions of class 1 integrons (integrase encoding gene and *qacE*Δ*1*/*sul1*) detected a 2.0-kb integron (GenBank accession number AM295980) in the strain 13.52. A walking sequencing strategy revealed two gene cassettes in the variable region of this integron, *aadB* and *catB3*, encoding resistance to aminoglycosides and chloramphenicol, respectively. This integron was identical to In-t1 carried by a *Salmonella enterica* serotype Typhimurium strain isolated from the stool of an infant hospitalized due to acute gastroenteritis at a university hospital in Tirana, Albania (12). Attempts to link the *qnrA* structure to the integron described above using long-extension PCRs with primers annealing in different positions of the variable region of the class 1 integron and *qnrA1* failed, suggesting that these two structures were separate.

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TABLE 1. Primers used for PCR and sequencing experiments in this study

Primer	Sequence (5′–3′)	GenBank accession no.
QNR-F	GATAAAGTTTTTCAGCAAGAGG	AY070235
QNR-R	ATCCAGATCGGCAAAGGTTA	AY070235
qnrFR	ATGGGCTCAGATCTCAGC	AY070235
qnrFF	CTGATAAAAGCGGCAGCG	AY070235
INTI1F	CCGTAGAAGAACAGC	AJ620678
att1	GCCTGTTCGGTTCGTAAGCT	AJ620678
QACR	GTTGCGATTACTTCG	AJ620678
QacFR	AGAGATGAGGGTTCGTTGCAAG	AY655485
sul1F	CTCACCGAGGACTCCTTC	AY655485
sul1FF	ACGTGCTGTCGAACCTTC	AY655485
sul1FR	GAAGGAGTCCTCGGTGAG	AY655485
aadBFF	TTTCAGGTCGCGATATGC	AJ009818
aadBR	AGACCTCAACCTTTTCCG	AJ009818
catB3F	TCAGAGTAGGCCGGTATAGC	AJ009818
catB3R	CGCCAATACTTATGCAGACC	AJ009818
catB3FF	ACAATGCCTCTGCTGTGCTC	AJ009818
catB3FR	AGCTATACCGGCCTACTCTG	AJ009818
CRF	CACT(A/T)CCACATGCTGT(G/T)(G/T)C	AY070235
CRR	CGCTTGAG(G/C)CGTTGC(A/G)(C/T)CC	AY070235
CR1FF	ACATGGACCAGGCTCAATTGTG	AY070235
CR1-FR	GCGACTTTCAACGCCATC	AY070235
DHA-1F	CACTGATTTCCGCTCTGC	AJ237702
DHA-1R	AGCCTGTGCAGCTTTGAC	AJ237702
CMY-1R	CCAACTGCGCCAGGATG	X92508
CMY-1F	CGACAATCCATCCTGTGG	X92508
CMY-2R	GGACAGGGTTAGGATAGC	X91840
CMY-2F	TCGTTATGCTGCGCTCTG	X91840
FOX-GenF	ATGCAACAACG(A/G)CGTGC	X77455
FOX-GenR	TCACTCGGCCAACTGACTCAG	X77455
aacA4F	TGCGATGCTCTATGAGTGGC	DQ303918
aacA4R	ATGTACACGGCTGGACCATC	DQ303918

TABLE 2. Susceptibility to selected antimicrobial agents of the *qnrA* donor (13.52), transconjugant (J53-13.52), and recipient (*E. coli* J53) strains

Antimicrobial agent	MIC (μg/ml) for:		
	Isolate 13.52	Transconjugant J53-13.52	*E. coli* J53
Nalidixic acid	256	128	4
Ciprofloxacin	8	2.0	0.047
Levofloxacin	8	1.5	0.064
Gatifloxacin	8	1.0	0.012
Ampicillin	>256	>256	8
Ampicillin/sulbactam	32/16	32/16	<4/2
Piperacillin-tazobactam	32	16	2
Cefoxitin	32	256	8
Ceftriaxone	8	8	0.047
Ceftazidime	64	128	0.25
Cefepime	0.5	0.5	0.06
Imipenem	≤0.025	≤0.025	≤0.025
Meropenem	≤0.025	≤0.025	≤0.025
Gentamicin	2	2	0.125
Amikacin	4	2	2
Kanamycin	32	128	8
Streptomycin	192	>1,024	>1,024
Chloramphenicol	>256	48	8

Mating experiments were carried out in liquid medium using a streptomycin-resistant *E. coli* J53 derivative strain. Transconjugants were selected on agar plates containing 10 μg of chloramphenicol/ml and 1,000 μg of streptomycin/ml. The presence of the *qnrA* and *catB3* genes in the selected transconjugants was confirmed by PCR and sequencing with specific primers, showing that both genes were present in the colonies obtained by conjugation. The J53-13.52 strain showed higher fluoroquinolone MICs (Table 2) than previously reported for *qnrA* transconjugants (fluoroquinolone MICs ranging from 0.25 to 1 μg/ml) (9). Due to this fact, ciprofloxacin, gatifloxacin, and levofloxacin MICs were confirmed by agar dilution according to the method of the Clinical and Laboratory Standards Institute (1). Sequencing of the *gyrA* and *parC* quinolone resistance determining regions, performed as previously described (3), revealed that these regions were identical in the donor, recipient, and transconjugant strains and did not contain mutations associated with quinolone resistance.

Mechanisms such as overexpression of the Acr efflux system and alteration in the outer membrane permeability were unlikely to contribute to the high fluoroquinolone resistance levels exhibited by the strain 13.52 and its transconjugant (4) since these mechanisms are chromosomally mediated and could not be transferred. In addition, the MICs for ciprofloxacin and nalidixic acid were not affected in the presence of the pump inhibitors, phenyl-arginine-β-naphthylamide, and reserpine (6; data not shown). Moreover, the outer membrane protein profile (2) was determined for the clinical (13.52), recipient (*E. coli* J53), and transconjugant strains. The profiles of the recipient and transconjugant J53-13.52 strains were identical (data not shown).

The high fluoroquinolone resistance level observed in the strain 13.52 and its transconjugant J53-13.52 could possibly be attributed to the association of *qnrA1* and a gene encoding the quinolone- and aminoglycoside-modifying enzyme AAC(6′)-Ib-cr, as recently described (9). However, PCR with primers targeting this resistance determinant failed to yield positive results with both strain 13.52 and its transconjugant J53-13.52. These results are in accordance with the amikacin susceptibility profile of the strain 13.52 and its transconjugant. Usually, AAC(6′)-Ib-cr-producing isolates are resistant to this compound as well as to kanamycin and tobramycin (9).

The mechanism of higher fluoroquinolone resistance conferred by the plasmid carried by strain 13.52, compared to that conferred by other Qnr-encoding plasmids (8. 10), could be attributed to a higher expression of *qnrA1* due to a higher plasmid copy number and/or to another yet-unknown plasmid-mediated resistant determinant. Further studies are under way to clarify this issue.

Since several β-lactamase genes have been found on *qnrA*-carrying plasmids (8, 10) and β-lactam resistance was transferred along with quinolone resistance to the transconjugant strain, the presence of β-lactamases was investigated in strain 13.52 and its transconjugant. The phenotypic detection of extended-spectrum β-lactamase using clavulanic acid was performed (1). No reduction in the ceftazidime MIC was observed in the presence of clavulanic acid. Primers for the genes encoding some plasmid-encoded class C β-lactamases, namely, the CMY, DHA, and FOX types, were used to screen for the presence of these genes by PCR (Table 1). PCR results and partial sequencing showed that the strain 13.52 and its transconjugant carried a $bla_{\text{FOX-5}}$-like β-lactamase gene. Notably, $bla_{\text{FOX-5}}$ was also detected in plasmid pMG252 (10).

Analysis of the plasmid content of the strain 13.52 and the transconjugant J53-13.52 was evaluated by electrophoresis on 0.8% agarose gel of the cleaved and intact plasmid preparation performed with a QIAGEN MIDI kit (QIAGEN, Hilden, Germany). According to the HindIII restriction profile, this strain

carried a single plasmid of approximately 41 kb (data not shown).

This is the first report of QnrA-producing *E. coli* strain in Brazil and Latin America and highlights the potential of plasmid-mediated fluoroquinolone resistance genes such as *qnrA1*.

The sequences determined in the present study are listed under GenBank accession numbers AM295981 for the *qnrA*-carrying *sul1*-type element and AM295980 for the copy of In-t1.

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